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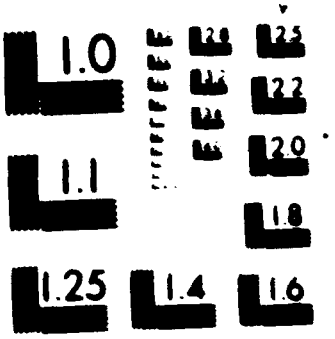
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We are developing strategies for computer-aided protein design. Our strategies emphasize simple geometric aspects of protein structure, and our computer program (PROTEUS) allows us to systematically test a very large number of alternative sequences and conformations. In preliminary work (funded by Procter & Gamble) we had added a disulfide bond to the N-terminal domain of lambda repressor. This increases the thermal stability by 8° C. Combining this disulfide bond with two glycine to alanine changes in alpha helix 3 variant that is 16° C more stable than the wild type proteins. Attempts are in progress to improve electrostatic interactions and the stacking of aromatic residues.

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Computer-Aided Design of Thermostable Proteins

We are developing methods for computer-aided protein design and testing these strategies by constructing thermostable variants of the lambda repressor. Repressor's DNA-binding domain normally denatures at 54 degrees Centigrade; our goal is to construct a variant that will bind normally to DNA and yet be stable to 100 degrees. In our first year, we have constructed a triple mutant that is stable to 70 degrees and binds DNA as well as the wild type protein.

Our fundamental goal is to develop methods for *de novo* protein design, and we are proceeding by treating the problem of protein design as an "inverted" version of the protein folding problem (Pabo, 1983). In the protein folding problem, one is given an amino acid sequence and must predict how this folds in three dimensions. Protein design can be approached in quite a different way - one can begin by chosing a folded arrangement of the polypeptide backbone and then try to pick an amino acid sequence that will stabilize this structure. "Inversion" eliminates the problem of predicting long-range interactions, since residues which will interact in the final tertiary or quaternary structure are already close in space when they are added to the prefolded backbone. One should be able to pick residues which will have favorable interactions with their neighbors.

We are developing a program, called PDB_PROTEUS, for computer-aided protein design. Our program uses simple geometric aspects of protein structure and frequently uses local coordinate systems so that the geometric relationships are easier to visualize (Pabo and Suchanek, 1986). There are many advantages to using a computer program: A program can easily check millions of possible sequences and conformations. Using a program also makes it easy to try several variations of a particular search strategy or to apply the same strategy to many different proteins. We have made extensive changes in our program over the past year. We have focussed on developing a library of FORTRAN subroutines that might eventually be shared by a number of laboratories. During the model building, these subroutines write intermediate results to disk files that use an extended version of the format used by Protein Data Base structure files. ["TEMP" records are added for points in space that may represent virtual atoms or guide points for the model building. "NOTE" records are used to keep lines of text that give information about the partial model.] Our disk files are used in much the same way that "blackboards" are used in some artificial intelligence programming (Hayes-Roth *et. al.*, 1983), and we expect to use AI programming strategies in future versions of PDB_PROTEUS.

Our first tests of PROTEUS were funded by the Procter & Gamble, and we began by writing a section that would systematically check the lambda repressor and find the best place to add a disulfide bond. Our search indicated that a disulfide bond could be introduced by changing Tyr88 to Cys. In collaboration with Robert Sauer's laboratory at M.I.T., we introduced this change: We found that the disulfide bond formed spontaneously *in vitro*, that it stabilized the protein against chemical or thermal denaturation and that the resulting covalent dimer bound DNA more tightly than the wild type N-terminal fragment(Sauer *et. al.*, 1986).

During the past year, we have tested to see whether the effects of multiple mutations are additive. (We expect that a set of changes will be needed to stabilize repressor to 100 degrees!) To test the effects of multiple mutations, we combined our disulfide mutant with two glycine to alanine changes in helix 3. Hecht and Sauer (1986) had shown that an Ala46Ala48 double mutant stabilizes the N-terminal domain of lambda repressor. We constructed the triple mutant, Ala46Ala48Cys88, and collaborated with Ernesto Friere to check the thermal stability in a scanning calorimeter. We found that the wild type protein denatured at 54 deg, the Cys88 mutant denatured at 62 deg, the Ala46Ala48 double mutant denatured at 62 deg, and the Ala46Ala48Cys88 triple mutant was stable to 70 deg!

We have written new sections for PDB_PROTEUS that allow us to search for places where new salt bridges might be added. We also have written section to search for places where aromatic residues could be added and make favorable contacts with existing aromatic residues (Burley and Petsko, 1985). Experimental tests of these predictions are currently in progress. (We have constructed a set of mutants and are purifying the proteins for calorimetry.)

Our goal (stabilizing repressor to 100 degrees) is ambitious enough that we will have to try every conceivable strategy for stabilizing repressor. We plan to develop model-building strategies that will let us search for places where we might: 1) improve the hydrophobic interactions and packing in the protein interior, 2) add metal-binding sites to stabilize the folded structure, and 3) add covalent crosslinks between side chains. As we proceed with this project, we also will check carefully to determine whether any irreversible chemical inactivation occurs at the higher temperatures. Our initial studies have tested binding at room temperature and then followed denaturation in a scanning calorimeter. We still must check that the protein is active until it actually unfolds, and we will try to directly measure binding at the higher temperatures.

References

Burley, S.K. and Petsko, G.A. (1985) Aromatic-Aromatic Interaction: A Mechanism of Protein Structure Stabilization **Science 229**, 23-28.

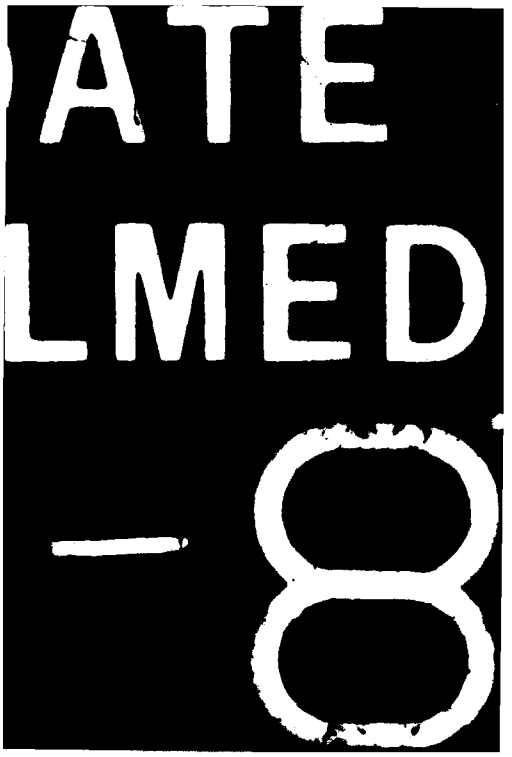
Hayes-Roth, F., Waterman, D.A., and Lenat, D.B., editors (1983) **Building Expert Systems** (Addison-Wesley Publishing Company; Reading, Mass.).

Hecht, M.H., Sturtevant, J.M., and Sauer, R.T. (1986) Stabilization of Lambda Repressor Against Thermal Denaturation by Site-Directed Gly to Ala Changes in Alpha Helix 3 **Proteins: Structure, Function, and Genetics 1**, 43-46.

Pabo, C.O. (1983) Molecular Technology: Designing Peptides and Proteins **Nature 301**, 200.

Pabo, C.O. and Suchanek, E.G. (1986) Computer-Aided Model-Building Strategies for Protein Design **Biochemistry 25**, 5987-5991.

Sauer, R.T., Hehir, K., Stearman, R.S., Weiss, M.A., Jeitler-Nilsson, A., Suchanek, E.G., and Pabo, C.O. (1986) An Engineered Intersubunit Disulfide Enhances the Stability and DNA Binding of the N-terminal Domain of Lambda Repressor **Biochemistry 25**, 5992-5998.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